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NOTES

Transformation of Bile Acids by *Eubacterium lentum*

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A group of fecal isolates identified as *Eubacterium lentum* elaborated 3α-, 7α-, and 12α-dehydrogenases and also an epimerizing enzyme(s) for the 3α-hydroxy group. The activities of the enzymes, however, were variably manifested according to the kind of bile acid substrate and the oxygen tension under which the reaction occurred.

In an earlier study (5), we isolated several strains of intestinal *Eubacterium lentum* which strongly 7α-dehydrogenated cholic acid and chenodeoxycholic acid to the respective 7-keto acids in growing cultures, concurrently with the conversion of their 3α-OH groups into the 3-keto and 3β-OH groups. Before our investigation, Midtvedt and Norman (11) reported the occurrence of these enzymatic reactions (7α-dehydrogenation, 3α-dehydrogenation, and 3α-epimerization) in cultures of six strains of the genus *Eubacterium* incubated with chenodeoxycholic acid. On the other hand, Macdonald et al. (9, 10) demonstrated 3α- and 12α-hydroxysteroid dehydrogenase activities in cell-free extracts from many strains of *E. lentum*, but they failed to reveal 7α-hydroxysteroid dehydrogenase activity in any of the test strains and did not mention epimerization of the 3α-hydroxy group.

In view of these somewhat conflicting findings, particularly on the 7α-dehydrogenase activity of *E. lentum*, and with the purpose of comparing the 3α-epimerization of this species with that of *Clostridium perfringens* previously reported (6), we reinvestigated the bile acid-transforming ability of our *E. lentum* strains.

Of the 7 strains of *E. lentum* previously isolated and characterized (5), 5 were tested. As the same reproducible results were obtained with all five of the strains, the data from a representative strain, HD-26, are presented in this paper. The transformation of bile acids by anaerobically growing cultures and by resting cells incubated under anaerobic or aerobic conditions was examined in the same manner as described for our previous study with *C. perfringens* (6). For cultural experiments, the strain was grown in a modified peptone-yeast extract broth (7) containing 100 μg of bile acid per ml and analyzed for bile acids after anaerobic incubation for 7 days (in an anaerobic jar under an atmosphere of pure 90% N_2 and 10% CO_2). For resting cell studies, cells were harvested by centrifugation from an overnight culture grown in the presence of 1% L-arginine (Nakarai Pharmaceutical Co.) to enhance the cell yield (no bile salt was added to the growth medium since no significant change in specific enzyme activity was observed in the presence or absence of bile salt in the medium). The cells collected were washed three times in 0.1 M sodium phosphate buffer at pH 7.5, and 40 mg (wet weight) of washed whole cells was incubated with 500 μg of bile acid in 3.0 ml of the same buffer for 4 h under anaerobic (in Thunberg tubes evacuated and filled with pure nitrogen) or aerobic (in L-shaped tubes continuously shaken in air) conditions. Free bile acids extracted from acidified samples (spent culture medium or incubated reaction mixture) were identified by the combined data from gas-liquid chromatography and mass spectrometry. Quantities of bile acids were determined from the area under the gas-liquid chromatographic peaks measured with an electronic integrator and expressed in percent composition after confirmation that the recovery of sample bile acids was compatible with the quantity of the original substrate bile acid. A detailed account of these procedures was given in our previous report (6).

The results with strain HD-26 are summarized in Table 1. Anaerobically growing cultures and anaerobically incubated resting cells showed essentially the same metabolisms. Chenodeoxycholic acid was 7α-dehydrogenated, yielding a large quantity of 7-ketolithocholic acid, and portions of both chenodeoxycholic acid and 7-ketolithocholic acid were epimerized as well as oxidized at the 3α-OH group. Ursodeoxycholic

TABLE 1. *Yield of metabolites formed from respective bile acids by anaerobically growing cultures of HD-26 and by resting cells of HD-26 incubated under anaerobic and aerobic conditions*

Substrate bile acid	Metabolite[a]	% metabolites formed by:		
		Anaerobic growing culture[b]	Anaerobic resting cells[c]	Aerobic resting cells[c]
Lithocholic acid	3α	NT	85	18
	3β	NT	4	2
	3κ	NT	11	80
Chenodeoxycholic acid	3α7α	16	10	6
	3β7α	2	2	1
	3κ7α	2	1	93
	3α7κ	63	69	0
	3β7κ	15	16	0
	3κ7κ	2	2	0
Ursodeoxycholic acid	3α7β	77	72	9
	3β7β	10	13	1
	3κ7β	13	15	90
Deoxycholic acid	3α12α	40	62	2
	3β12α	7	10	0
	3κ12α	10	3	6
	3α12κ	32	20	31
	3β12κ	5	3	6
	3κ12κ	5	2	55
Cholic acid	3α7α12α	16	9	2
	3β7α12α	2	2	0
	3κ7α12α	6	0	0
	3α7κ12α	60	71	0
	3β7κ12α	16	17	0
	3κ7κ12α	0	1	0
	3α7α12κ*	0	0	17
	3β7α12κ*	0	0	4
	3κ7α12κ*	0	0	77

[a] In terms of substituents in 5β-cholanoic acid (α, α-OH; β, β-OH; κ, keto group; *, presumptive identification).

[b] Seven-day cultures grown in the presence of 100 μg of the indicated bile acid per ml were analyzed; the average of duplicate tests is shown. NT, Not tested.

[c] Reaction mixtures containing 40 mg of washed cells incubated with 500 μg of bile acid for 4 h under anaerobic or aerobic conditions were assayed. The average of duplicate tests is shown.

acid, the 7β-hydroxy epimer of chenodeoxycholic acid, was not affected at C-7, but was readily oxidized and epimerized at the 3α-OH group, a finding analogous with that for lithocholic acid, which has only the 3α-OH group. Cholic acid was metabolized like chenodeoxycholic acid; i.e., it was readily oxidized to 7-ketodeoxycholic acid through 7α-dehydrogenation, attended by the oxidation and epimerization of the 3α-OH groups in both cholic acid and 7-ketodeoxycholic acid. The 12α-OH group in cholic acid remained unaffected throughout the incubation. Deoxycholic acid, which lacks a hydroxy substituent at C-7, was readily dehydrogenated at its 12α-OH group, and 3-keto and 3β-OH derivatives were formed from both deoxycholic acid and the 12-keto product. It is apparent from these data that the organism constitutively elaborates the whole series of 3α-, 7α-, and 12α-dehydrogenases, and that the 3α-dehydrogenase activity of this organism is closely associated with the epimerization of the concerned hydroxy group.

It should be noted in this connection that the demonstration of 12α-dehydrogenase activity depended upon the kind of substrate bile acid. Although the 12α-OH in deoxycholic acid was oxidized, the same OH group in cholic acid was not metabolized by growing or resting cells under anaerobic conditions. Steric hindrance of 12α-dehydrogenation by the presence of a hydroxy group at C-7 has been suggested (1, 3), but, as shown below, cholic acid was readily 12α-dehydrogenated by the same cell preparation

when incubated aerobically, with suppressed 7α-dehydrogenation. Not the mere presence of an intact OH group at C-7 but rather the active processing of the 7α-OH group under anaerobic conditions seems to prevent the 12α-dehydrogenation of cholic acid. In any case, there seems to exist the following incompatibility between the dehydrogenation at C-7 and at C-12 in cholic acid: extensive 7α-dehydrogenation under anaerobic incubation, and only 12α-dehydrogenation in an aerated system.

3β-Epimers were invariably formed from all of the 3α-hydroxy bile acids and consistently associated with 3α-dehydrogenation. The results are in good accord with those obtained with *C. perfringens* (6), and the 3-keto and 3β-hydroxy convertants formed by these two kinds of organisms were quite comparable in gas-liquid chromatographic and mass spectrometric features (4). In contrast to *C. perfringens*, which did not affect the hydroxy groups other than at C-3, *E. lentum* oxidized the 7α-OH and 12α-OH groups in addition. There have been short communications by Midtvedt and Norman (11) and Bokkenheuser et al. (2) concerning the formation of 3β-epimers by *E. lentum*, but a detailed account is given for the first time in this report.

Interestingly, aeration of resting cells completely inhibited the dehydrogenation of the 7α-OH group in both cholic acid and chemodeoxycholic acid, whereas the dehydrogenation at C-3 or C-12 or both was significantly enhanced (Table 1 and Fig. 1). As a rule, dehydrogenating keto formation is stimulated by aeration, presumably because of the abundant supply of oxygen as an effective terminal electron acceptor or by establishment of a higher redox potential of the medium needed for the enzyme to function efficiently. The 7α-dehydrogenation of this bacterium was exceptional in this respect. It may be assumed that the preferable 3-keto formation could sterically hinder 7α-dehydrogenation (8). However, the preference of oxidation of the 3α-OH (or 12α-OH or both groups) to that of the 7α-OH group by the *E. lentum* cells which contain all of the concerned dehydrogenases itself implies differences in oxygen relation among these dehydrogenase activities.

Such a specific requirement for an anaerobic environment of the 7α-dehydrogenase activity of this bacterium seems to explain the discrepancy between the results of Midtvedt and Norman (11) and those of Macdonald et al. (9, 10); the former authors demonstrated 7α-dehydrogenase activity in anaerobic cultures of *Eubacterium*, whereas the latter workers did not detect the activity in cell-free preparations from *E. lentum* which might have been treated under aerobic conditions. Epimerizing conversion of the 3α-OH group, which presumably involves a reductive process from a 3-keto to a 3β-OH, was suppressed by aerobic incubation. The suppression was not necessarily complete but was more marked than that by *C. perfringens* resting cells tested under similar conditions (6).

In summary, the *E. lentum* strains tested in this study possessed 3α-, 7α-, and 12α-dehydrogenases and 3α-epimerizing enzyme(s) but the manifestation of the activity of these enzymes was influenced by such experimental conditions

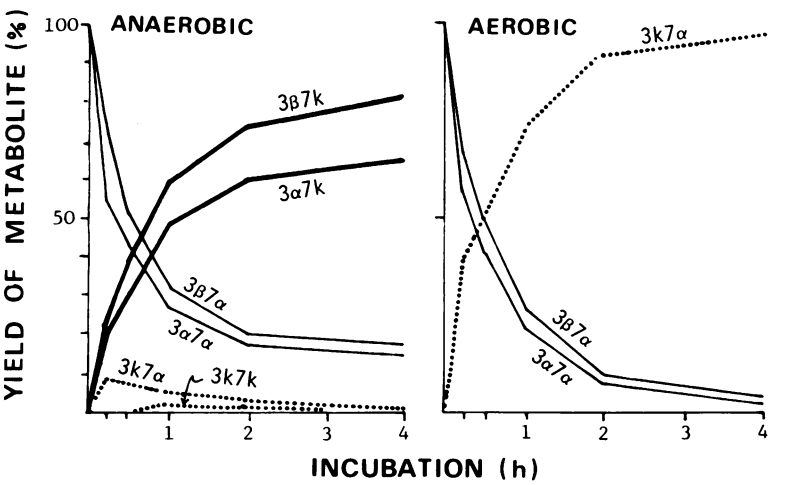

FIG. 1. *Time course of transformation of chenodeoxycholic acid by resting cells of HD-26 under anaerobic and aerobic conditions. Forty milligrams of resting cells was incubated for the specified periods with 500 μg of chenodeoxycholic acid under anaerobic and aerobic conditions. For notation of bile acids, see Table 1, footnote a. 3β-Epimeric bile acids are presented cumulatively to 3α-analogs.*

as the kind of substrate and the oxygen supply to the medium.

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